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A possible new relay for tongue thermal sense in the dorsal margin of the trigeminal principal nucleus in rats

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Abbreviations: BC, brachium conjunctivum; PB, parabrachial nucleus; PV, trigeminal principal nucleus; SpVe, caudal subnucleus of the spinal trigeminal nucleus; SpVi, interpolar subnucleus of the spinal trigeminal nucleus; SpVo, oral subnucleus of the spinal trigeminal nucleus; VPM, posteromedial ventral thalamic nucleus; VPMpc, parvicellular part of the posteromedial ventral thalamic nucleus; WGA-HRP, wheat germ agglutinin-conjugated horseradish peroxidase
ABSTRACT

Neurons responding to innocuous thermal stimulation of the anterior tongue were newly identified in the dorsal margin of the trigeminal principal nucleus (PV). Connections of the dorsal margin of the PV were neuroanatomically identified using wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) with electrophysiological techniques. Injection of WGA-HRP into the dorsal margin of the PV demonstrated anterograde labels distributed from the most dorsomedial portion of the posteromedial ventral nucleus (VPM) to the dorsolateral portion of the parvicellular part of the VPM, and retrograde labels in the superficial layers of the caudal subnucleus of the spinal trigeminal nucleus (SpVc). Injection of WGA-HRP in the most dorsomedial portion of the VPM demonstrated retrogradely labeled cells in the dorsal margin of the PV as well as in the superficial layers of the SpVc. Tracer injection into the superficial layers of the SpVc resulted in anterograde labels in the dorsal margin of the PV as well as in the dorsomedial portion of the VPM. These findings show that neurons in the PV respond to innocuous thermal stimulation of the trigeminal field and suggest that the dorsal margin of the PV is a possible new relay for the tongue thermal sense, receiving thermal information from the superficial layers of the SpVc, the primary thermal relay for the trigeminal field, and passing the information to the thalamic relay.

Keywords: Trigeminal principal nucleus
Wheat germ agglutinin-conjugated horseradish peroxidase
Thermal responses
Posteromedial ventral thalamic nucleus
Caudal subnucleus of the spinal trigeminal nucleus

Section: Sensory and Motor Systems
1. Introduction

Thermal information from the trigeminal field is conveyed by the trigeminal nerve to the caudal subnucleus of the spinal trigeminal nucleus (SpVc), which forms the primary thermal relay transmitting thermal information to the thalamic relay in many animal species (Bade et al., 1979; Burton et al., 1979; Craig and Dostrovsky, 1991; Davies et al., 1985; Dickenson et al., 1979; Fukushima and Kerr, 1979; Heinz et al., 1990; Hutchison et al., 1997; Iwata et al., 1992; Kemplay and Webstar, 1989; Landgren, 1957, 1960; Sumino and Dubner, 1981). Thermal information from the anterior two-thirds of the tongue is sent to the SpVc through the lingual nerve, a branch of the trigeminal nerve (Kosar and Schwartz, 1990; Lundy and Contreras, 1994). The physiological properties of the SpVc neurons responsive to innocuous thermal stimulation of the tongue were fully examined in rats (Hutchison et al., 1997; Zanotto et al., 2007). The anterior two-thirds of the tongue is also innervated by the chorda tympani nerve, a branch of the facial nerve, which contains many thermosensitive and thermoreceptive fibers as well as taste fibers (Ogawa et al., 1968). Thermosensitive neurons are also present in the central gustatory relay nuclei and the gustatory cortex (Kosar et al., 1986; Kosar and Schwartz, 1990; Ogawa et al., 1988, 1990; Scott and Perrotto, 1980; Yamamoto et al., 1980), indicating that the gustatory ascending system carries thermal as well as taste information. An investigation of thermosensitive neurons in the parabrachial nucleus, the secondary gustatory relay (Hayama et al., 1987; Hayama and Ogawa, 1987; Norgren and Pfaffmann, 1975), and its surrounding regions also detected many neurons responsive to innocuous thermal stimulation of the tongue in the dorsal margin of the trigeminal principal nucleus (PV), where neurons responsive to thermal stimulation of the trigeminal field had not been previously reported.

The present study examined the possibility that the dorsal margin of the PV is another relay for the tongue thermal sense by neuroanatomical identification of the
neuronal connections using wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP).

2. Results

2.1. Thermal responses in the dorsal margin of the PV

The physiological properties of neurons in the dorsal margin of the PV were examined by electrophysiological recording of neural activity during application of thermal and tactile stimuli to the anterior tongue in three rats. The stimuli were given during electrode advancement at intervals of 100 µm in depth. Spontaneous multiunit activity was often detected immediately before the electrodes advanced deep into the regions where neurons were activated by tactile stimulation of the oral cavity or perioral area. The spontaneous activity was suppressed by application of warm water, recovered soon after discontinuation, and was potentiated by application of cold water. No phasic potentiation of the activity was induced by application of warm water, indicating that the responses were thermal and not mechanical. Figure 1 shows an example of reconstruction of recording sites in the dorsal margin of the PV and its vicinity in one animal. Spontaneous neural activity at one site was suppressed by warm stimulation and phasically potentiated by cold stimulation (Fig. 1C), indicating that the multiunit activity was cold-sensitive. The recording site was located in or near the dorsal boundary of the PV (Fig. 1B). All recording sites of thermal responses in the three animals were superimposed on several coronal brain sections (Fig. 2). Thirteen of the 15 thermosensitive responses were located on the dorsal margin of the PV and two responses were located in the parabrachial nucleus (PB). All 15 thermosensitive multiunit activity responses were suppressed by application of warm water and potentiated by application of cold water.
2.2. Connections of the dorsal margin of the PV

Eleven rats received WGA-HRP in the dorsal margin of the PV and its surroundings. Five rats (Cases 1-5) received a moderate amount of tracer immediately after recording the neural responses to thermal stimulation of the tongue with a tracer-containing electrode. Six rats received tracer injection at sites adjacent to the thermosensitive area. Two of these 6 rats received tracer injection immediately after recording neural responses to tactile stimulation of the lower incisors (Case 6) and the tongue (Case 7). The remaining four rats (Cases 8-11) received tracer injection in adjacent areas mediodorsal to the thermosensitive area; no neural responses were recorded with a tracer-containing electrode except for one rat (Case 9) who received tracer at a site where neural activity synchronized with respiratory movement was unexpectedly recorded. All injection sites were histologically centered in the dorsal portion of the PV and its surroundings (Fig. 2). The injection sites in Cases 1-5 were centered on the dorsal boundary of the PV where many thermosensitive cells were identified electrophysiologically. The injection site in Case 6 was centered on the dorsomedial portion of the PV at a more caudal level. The injection site in Case 7 was recognized as a dorsoventrally elongated area in the dorsal PV at a more caudal level, where responses to tactile stimulation of the tongue were observed all along the electrode track from the dorsal margin of the PV. The injection sites of the remaining 4 rats (Cases 8-11) were centered in the lateral tip area of the brachium conjunctivum (BC) and its surroundings which correspond to the external medial and external lateral subnuclei of the PB. Anterograde and/or retrograde labels were observed throughout the brain following tracer injection. Labels in the thalamus and the brainstem are described below.

2.2.1. Labels in the thalamus

Cases 1-5 demonstrated dense anterograde labels commonly located in areas extending
from the most dorsomedial portion of the posteromedial ventral nucleus (VPM) to the
most dorsolateral portion of the medial extension of the VPM, that is the parvicellular
part of the VPM (VPMPc), with clear contralateral dominance. This area coincides well
with the previously identified area containing many neurons responsive to thermal
stimulation of the tongue (Hayama and Ogawa, 2003). The distributions of the labels
varied slightly in the five rats. Figure 3 shows thermal responses recorded at the
injection site and subsequent label distributions in the thalamus in Case 3. The
distribution of dense labels in the medial portion of the contralateral VPM extended
rostrally to levels rostral to the rostral pole of the VPMPc, that is the level of Fig. 3Ca,
in all rats. However, the rostral labels were sparser in two rats (Cases 1 and 3) than in
the other rats (Cases 2, 4, and 5). Cases 1 and 3 received tracer injections in slightly
more dorsal portions of the dorsal margin of the PV. Sparse labels were also observed in
the whole area of the VPMPc, the central medial and mediodorsal thalamic nuclei, and
the caudal portion of the parafascicular nucleus in all five rats.

Case 6 showed anterograde labels located contralaterally in a strip along the
dorsal surface of the medial portion of the medial lemniscus. No labels were seen in the
VPMPc or in the dorsomedial portion of the VPM. Case 7 had dense anterograde labels
located contralaterally in the medial portion of the VPM; the labels were not confined to
the dorsomedial portion. The labels were denser in levels rostral to the VPMPc and
sparse in the VPMPc.

Cases 8-11 showed quite different distribution patterns of anterograde labels
in the thalamus. The labels were commonly observed in the bilateral VPMPc and/or
peripheral portions of the nucleus. However, the labels were not observed in the VPM
except for sparse labels in the medial portion of the VPM in Case 8. Sparse labels were
also seen in the parafascicular and subparafascicular thalamic nuclei, the central medial
thalamic nucleus, and areas between the bilateral VPMPc.
These findings suggest that thermosensitive neurons in the dorsal margin of the PV project to the most dorsomedial portion of the VPM and the most dorsolateral portion of the VPMpc.

2.2.2. Labels in the brainstem
Cases 1-5 showed ipsilateral distributions of retrograde and anterograde labels in the brainstem. Moderate numbers of retrogradely labeled cells were seen in the dorsomedial portion of the SpVc and in the paratrigeminal nucleus. A few retrograde labels were found in the dorsomedial portions of the oral (SpVo) and interpolar subnuclei (SpVi) of the spinal trigeminal nucleus in all rats, and in the lateral portion of the rostral solitary tract nucleus, the dorsal column nucleus, and the vestibular nucleus in one rat each. The labels in the SpVc were distributed in the superficial layer (laminae I-II) of the dorsomedial portion of the subnucleus (Fig. 3B); the distribution area was elongated to about 1 mm rostrally and 1-2 mm caudally from the obex. A few labels were also found in the deep layers (laminae III-IV) of the subnucleus in three rats. Anterograde labels were also seen in the dorsomedial portion of the SpVo, SpVi, and SpVc, the solitary tract nucleus, the facial motor nucleus, the hypoglossal nucleus, and the superficial layer of the dorsal cochlear nucleus.

Cases 6 and 7 showed similar distributions of retrograde and anterograde labels to Cases 1-5, except that the labels in the deep laminae were denser than those in the superficial laminae in the SpVc. Cases 8 and 9 had similar distributions of retrograde and anterograde labels to Cases 1-5, except that the labels in the SpVc were not confined to the dorsomedial region of the nucleus but were also located in the whole extent of the superficial layer. Case 11 had no labels in the superficial layer but did have labels in the deeper layers of the SpVc. Case 10 showed no labels in the brainstem, perhaps because the amount of injected tracer was small.
2.3. Connections of the thalamic area responding to thermal stimulation of the tongue

Retrograde labels were explored on the dorsal margin of the PV and its surroundings after tracer injection into the VPM or VPMpc in areas of neuronal responses to thermal (Cases 12-14), tactile (Cases 15-17), and taste (Cases 18-20) stimulation of the tongue as identified with the tracer-containing electrode. Since multiunit thermal responses could not be identified, tracer was injected into the sites showing background neuronal activity decreased on warm stimulation and increased on cold stimulation, although multiunit responses were identified to taste and tactile stimulation. All injection sites were histologically centered in the VPMpc and the medial portion of the VPM, and were superimposed on coronal sections of three levels of the thalamus (Fig. 4). Injection centers were located in the dorsomedial portion of the VPM and the dorsolateral portion of the VPMpc in Cases 12-14. Injection centers were located in the medial portion of the VPM adjacent laterally to the thermal responsive zones in Cases 15-17, and in the VPMpc adjacent medially to the thermal regions in Cases 18-20.

Many retrograde labels were observed in the dorsal portion of the contralateral PV in Cases 12-14. A few retrograde labels were also observed in the bilateral PBs and in the dorsal margin of the ipsilateral PV in Case 14. Many retrograde labels were seen in the dorsal portion of the contralateral PV in Cases 15-17. However, the labels were distributed in more medial, ventral, and caudal regions than in Cases 12-14; many labels were found in more caudal sections than the level of the genu of the facial nerve. A few retrograde labels were seen ventrolateral to the lateral tip area of the BC along the dorsal boundary of the PV in the contralateral side. Many retrograde labels were seen in the bilateral PBs and in the areas ventrolaterally adjacent to the lateral tip area of the BC along the dorsal boundary of the PV in the contralateral side in
Cases 18-20. A few retrograde labels were also found in the dorsomedial portion of the PV in Case 19. Examples of distributions of retrograde labels (Cases 12, 15, and 18) are shown in Fig. 5. Retrograde labels in the dorsal portion of the PV in Case 12 are shown in Fig. 6A. These findings coincide well with the distribution patterns of anterograde labels resulting from tracer injections into the dorsal portions of the PV and its vicinity as shown above.

Many retrograde labels were found in the superficial layer (laminae I-II) of the SpVc in Cases 12-14 (Fig. 6B). The distribution extended rostrocaudally to about 1 mm around the obex, which was similar to that of the retrograde labels seen after tracer injection into the dorsal margin of the PV. Similar distribution patterns of retrograde labels in the SpVc were observed in Cases 15-19 but no labels were found in Case 20.

Dense anterograde and retrograde labels were seen in the insular cortex, specifically the dorsal half of the dysgranular insular to granular insular cortices, in Cases 12-14. The labels were distributed in a rostrocaudally elongated area with the densest labels at about 1.5-2.0 mm rostral to the rostral pole of the bed nucleus of the anterior commissure. Anterograde labels were densest in a deep portion of cortical layer II/III and layer IV, and sparse labels were also found in all other layers. Retrograde labels were observed in cortical layers V and VI. The distribution pattern of the labels was the same in the three rats. Dense anterograde and retrograde labels were seen in the granular insular cortex in Cases 15-17, and anterograde and retrograde labels were found in the dysgranular insular cortex in Cases 18-20.

2.4. Connections of thermally responsive regions in the SpVc

These anatomical experiments suggest that neurons in the superficial layer of the SpVc project their axons to the dorsal boundary of the PV, and to the most dorsomedial region of the VPM and the dorsolateral portion of the VPMpc. Therefore, anterograde labels
were explored in those regions after tracer injection into the dorsomedial regions in the SpVc. Spontaneous multiunit activity was often found immediately after the electrodes were advanced into the dorsal surface of the caudal medulla at around the obex level in four rats. This spontaneous activity was suppressed by warm water and potentiated by cold water, indicating that the multiunit activity was cold-sensitive. Thermosensitive multiunit responses were observed in 23 recording sites from the four rats; all multiunit activity was cold-sensitive. The recording sites were superimposed on the dorsal surface of the caudal medulla (Fig. 7A), and were distributed on a region extending mediolaterally from 1.5 to 2.5 mm from the midline, and rostrocaudally by about 1.5 mm; the distribution pattern was similar to that of the retrograde labels seen after tracer injection into the dorsal boundary of the PV or in the thalamic area shown above. WGA-HRP was injected into two sites responsive to thermal stimulation of the tongue in 2 of the 4 rats (Fig. 7B and C) immediately after confirming thermal responses with a tracer-containing electrode. Histological examination showed that injected tracer was confined to the superficial layer of the SpVc (Fig. 8A). Dense anterograde labels were found ipsilaterally in the dorsal boundary of the PV (Fig. 8B). Dense labels were also observed caudally in the central portion of the dorsal PV and rostrally in a region around the lateral tip of the BC, and sparse labels were seen throughout the PB. Dense anterograde labels were distributed contralaterally in the most dorsomedial portion of the VPM and the most dorsolateral portion of the VPMpc (Fig. 8C). Distributions of the anterograde labels in the PV and in the thalamus were similar in the two rats.

3. Discussion

3.1. Thermosensitive multiunit responses

Multiunit rather than single unit activity responding to thermal stimulation of the tongue was explored in the thalamus, the PV, and the SpVc to quickly map thermosensitive
regions. Thalamic thermosensitive neurons showed spontaneous activity caused by reduced surface temperature of the anterior tongue because the tongue was positioned ventrolaterally with its tip extended from the oral cavity (Hayama and Ogawa, 2003). Therefore, multiunit activity with spontaneous activity was examined first by warm water application to quickly identify the thermosensitive neurons. All thermosensitive multiunits in the PV and SpVc were cold-sensitive, probably because cold-multiunit activity was easily identified because of the spontaneous activity, and thermal neurons in the SpVc, the primary thermal relay, are predominantly cold-sensitive (Hutchison et al, 1997).

3.2. Thermal responses on the dorsal margin of the PV
Multiunit activity responsive to innocuous thermal stimulation of the anterior part of the tongue was often encountered on the dorsal margin of the PV. The PV is the first relay for the trigeminal field, which receives trigeminal afferent fibers from the facial, perioral, and oral areas, and projects to the contralateral ventrobasal thalamus (Kemplay and Webster, 1989; Takemura et al., 1991; Guy et al., 2005). The present finding is the first evidence that neurons in the trigeminal nucleus other than the SpVc responded to innocuous thermal stimulation. Whether thermal information from the trigeminal fields other than the tongue is also processed in the dorsal margin of the PV is an important issue to be examined in the future.

3.3. A possible new central relay for thermal information from the tongue
The present experiments using anterograde and retrograde labeling methods showed that thermally responsive regions in the dorsal margin of the PV project contralaterally to the most dorsomedial portion of the VPM and the most dorsolateral area of the VPMpc, where the thalamic neurons responding to thermal stimulation of the anterior
tongue are located (Hayama and Ogawa, 2003). The thermally responsive areas in the PV also received afferents ipsilaterally from the superficial portion of the dorsomedial portion of the SpVc, the primary thermal relay in the trigeminal field which sends thermal information to the thalamic relay (Hutchison et al., 1997; Craig and Dostrovsky, 1991). Tracer was injected into the thalamus, PV, and SpVc immediately after recording thermal responses with a tracer-containing electrode, although multiunit thermal responses could not be recorded in the thalamic thermal area where increase or decrease in background neuronal activity was observed after cold or warm stimulation, respectively. These findings suggest that a central pathway from the SpVc to the thalamus through the dorsal portion of the PV conveys thermal information from the tongue. Furthermore, tracer was injected into neighboring areas of the thermal zone in the PV, that is regions responding to tongue or teeth tactile stimulation adjacent caudally to the thermal zone, and the lateral tip area of the BC. Comparison of the distribution patterns of anterograde labels in the thalamus also suggested that the dorsal margin of the PV projects to the most dorsomedial portion of the VPM and the most dorsolateral area of the VPMpc, the tongue tactile region in the PV located caudally to the thermal area projects to adjacent thalamic areas lateral to the thalamic thermal area, and the lateral tip area of the BC projects mainly to the VPMpc. The distribution patterns of retrograde labels in the PV and its surroundings following tracer injections into the thermal region in the thalamus and its surroundings, that is the taste and tongue tactile regions, also support these proposed connection patterns between the PV and the thalamus. Therefore, we propose that the thermally responsive area in the dorsal margin of the PV is a new relay for the tongue thermal sense which receives thermal information from the SpVc and sends the information to the thalamic relay. However, the present method of tracer injections coupled with electrophysiological recordings cannot exclude the possibility that tracer molecules injected into the thermal regions
might be incorporated by neurons of unknown function adjacent to the thermosensitive neurons and transported to other brain regions. Therefore, our hypothesis must be further examined by other experimental techniques, such as intracellular single cell recording and labeling.

Trigeminal afferent fibers expressing the P2X3 receptor extend into the dorsal portion of the PV in addition to other trigeminal subnuclei; about 90% of trigeminal afferent fibers expressing P2X3 receptor are unmyelinated and the remaining fibers are thinly myelinated (Kim et al., 2008). Therefore, the thermal area in the dorsal margin of the PV might receive thermal information directly from the trigeminal afferent fibers as well as from the SpVc, since innocuous thermal information is passed by unmyelinated or thinly myelinated fibers (Iriuchijima and Zotterman, 1960; Hensel et al., 1960; Perl, 1968). The present anatomical evidence indicates that thermal information from the tongue is sent to the insular cortex at about 1.5-2.0 mm rostral to the bed nucleus of the anterior commissure, which is known to be the cortical taste area containing neurons responding to taste, thermal, and tactile stimulation of the tongue (Kosar et al., 1986; Ogawa et al., 1990; Yamamoto et al. 1980).

4. Experimental procedure

4.1. Surgery

All surgical procedures were conducted following the Guidelines for Animal Treatment issued by our institution and by the Physiological Society of Japan. Albino Sprague Dawley rats, male and female, weighing 250-450 g were anesthetized intraperitoneally with amobarbital sodium (80 mg/kg). Animals were maintained areflexic by intraperitoneal injection when necessary of amobarbital sodium in experiments exploring neuronal activities in the dorsal portion of the PV or urethane (20% solution) in experiments on the thalamus and SpVc. The animals were mounted on a stereotaxic...
instrument. A part of the skull was removed to search for neural activity and to inject WGA-HRP. A portion of the parietal bone and/or a rostral portion of the interparietal bone were removed to allow the recording of neural activity in the thalamus or the parabrachial region and its surroundings. A part of the occipital bone and a portion of the neck muscle were removed to expose the dorsal surface of the caudal medulla for evaluation of neural activity in the SpVc. Rectal temperature was maintained at about 37°C with a water heater. Electrocardiographical monitoring was continued throughout the experiment.

### 4.2. Electrophysiology

To identify neuronal activity in the parabrachial region and its surroundings, a glass micropipette (tip diameter 1-3 µm), filled with 0.5 M sodium acetate containing 2% pontamine sky blue, was inserted into the occipital cortex or the cerebellum rostrocaudally at 5-15° from the vertical. An indifferent silver wire electrode was placed on the neck muscle. The mouth of the subject animal was pulled open wide with a weight attached to the lower incisors with a piece of thread. The tongue was positioned ventrolaterally with its tip extended from the oral cavity. Thermal, tactile, and taste stimuli were applied to the tongue anterior to the intermolar eminence or perioral areas to provoke neuronal activity; all three types of stimulation were used to identify neuronal activity in the thalamus, thermal, and tactile stimulation were used to identify activity in the PV and its surroundings, and only thermal stimulation was used to identify activity in the SpVc. Thermal stimulus consisted of distilled water at 20°C or 40°C applied with a pipette to the anterior tongue. Tactile stimulation was conducted with a glass rod. Taste stimulus used 0.1 M NaCl solution applied with a pipette to the anterior tongue since the thalamic neurons responding best to the NaCl are more numerous than neurons responding best to other taste qualities (Nomura and Ogawa,
1985). Multiunit activity was passed to a preamplifier and displayed on a cathode ray oscilloscope. Impulse discharges were then passed to a spike counter with window discriminator function (DSE325P; Dia Medical Systems Co., Ltd., Tokyo, Japan) and finally recorded on an ink-trace oscillograph to construct peristimulus time histograms. Some of the recording sites were marked by electrophoretic deposition of dye from the recording electrodes. After the experiment, the animal was deeply anesthetized and perfused intracardially with 10% formalin solution in 0.1 M-phosphate buffer (pH 7.4). The recording sites of the thermosensitive neurons were histologically reconstructed on coronal brain sections (50 µm thickness).

4.3. Neuroanatomy

Prior to tracer injection, the stereotaxic coordinates of injection loci were identified electrophysiologically. A glass micropipette (tip diameter 1-3 µm) filled with 2 M NaCl solution was inserted into the dorsal margin of the PV, the thalamic area for tongue thermal sense (Hayama and Ogawa, 2003), or the dorsal portion of the SpVc. The procedures for thermal stimulation and recording of multiunit activity were as described above. After recording the thermal responses of neurons, the electrode was replaced with a micropipette (tip diameter 10-20 µm) containing about 4% WGA-HRP (Toyobo, Osaka, Japan) dissolved in 0.1 M KCl and 0.05 M Tris buffer (pH 8.0) solution. The micropipette electrodes were inserted into the brain to confirm the location of the electrode tip at the sites responsive to thermal stimulation, and then tracer was injected by applying positive direct current pulses (1-3 µA current, 250 ms duration, 2 Hz) over 3-15 min. After about 24 h, the animals were deeply anesthetized with an overdose of anesthetic and perfused through the heart with about 50-100 ml of cold phosphate buffer (0.1 M, pH 7.3) followed by 200-300 ml of cold phosphate-buffered 10% formalin solution. The brain was removed from the skull and kept for about 24 h in cold...
phosphate buffer containing 20% sucrose without postfixation. Coronal sections (50 µm) were prepared using a freezing microtome. Alternate coronal sections were treated for peroxidase activity (Mesulam, 1978) and for Nissl staining. Reaction products due to peroxidase activity were stabilized with ammonium molybdate (Fujii and Kusama, 1984). Brain sections treated for peroxidase activity, with or without light counterstaining, were examined under a light microscope using bright-field or polarized optics after a conventional dehydration procedure.

Photomicrographs of brain sections were taken with a digital microscope camera (PDMC; Nippon Polaroid KK, Tokyo, Japan). Photomicrographs of some brain sections were taken with a film type camera (HFX-DX; Nikon, Tokyo, Japan) and the prints were then scanned with an image scanner (GT-9500; Epson Singapore Pte Ltd., HarbourFront Tower 1, Singapore). The brightness and contrast of the digital images were adjusted with Adobe Photoshop (Adobe Systems, San Jose, CA). Final preparation of the figures was accomplished with Adobe Illustrator (Adobe Systems). Estimation of the anteroposterior level of brain sections and the delineation of nuclei were mainly based on Paxinos and Watson (1986).
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**LEGENDS**

Fig. 1 – Thermal responses in the dorsal margin of the PV. (A) Photomicrograph of a Nissl-stained coronal section showing the recording sites (dye marks) of neurons responding to thermal stimulation of the anterior tongue. The coronal level is -9.6 mm from the bregma. Scale, 0.5 mm. (B) Reconstruction of recording sites of neurons in the dorsal portion of the PV at the coronal level shown in (A). Numbers 1-5 indicate electrode tracks. Solid circles, recording sites of thermal responses; open circles, tongue tactile responses; solid squares, responses to tactile stimulation of the upper or lower lips; open squares, responses to tactile stimulation of the buccal pad; open diamonds, respiration-related activity; solid diamonds, periodontal mechanical responses. Two solid circles indicated by arrows correspond to the sites of dye marks (arrows) in (A). (C) Peristimulus time histogram of neuronal responses to thermal stimulation. The recording site of the neurons is the lower of the two solid circles along the electrode track 3 in (B). W and C show application of warm (40°C) and cold (20°C) water. BC, brachium conjunctivum; MoV, motor trigeminal nucleus; PV, trigeminal principal nucleus.

Fig. 2 – Composite map of recording sites of the 15 thermal neurons (small solid circles) and 11 centers of WGA-HRP injection (large white, hatched, and gray circles). Recording sites from three rats are collectively plotted on representative coronal sections of the PV. The coronal levels are located -9.2 to -10.0 mm from the bregma and shown in the lower portion of each section. Numbering of the large circles indicates case number; white and hatched circles show the injection centers for thermal and tactile regions. BC, brachium conjunctivum; IC, inferior colliculus; LC, locus coeruleus; mcp, middle cerebellar peduncle; MeV, mesencephalic trigeminal nucleus;
meV, mesencephalic trigeminal tract; MoV, motor trigeminal nucleus; PV, trigeminal principal nucleus; vsc, ventral spinocerebellar tract; 7n, facial nerve.

Fig. 3 – Injection site of WGA-HRP in the PV and resulting anterograde and retrograde labels in Case 3. (Aa) Injection center of WGA-HRP indicated with a white circle. The coronal level is located -9.8 mm from the bregma. (Ab) Thermal responses recorded at the site with a tracer-containing electrode immediately before injection. W and C indicate application of warm (40°C) and cold (20°C) water. (B) Resulting retrograde labels (arrowheads) in the superficial portion of the SpVc. (Ca-Cc) Resulting anterograde labels at three levels (rostral to caudal, -3.6 to -4.0 mm from the bregma) of the VPM and VPMpc and its surroundings. The right side is ipsilateral to the injection site. Scale, 0.5 mm in Aa, 0.05 mm in B, and 0.2 mm in C. BC, brachium conjunctivum; CM, central medial thalamic nucleus; fr, fasciculus retroflexus; mcp, middle cerebellar peduncle; ml, medial lemniscus; OPC, oval paracentral thalamic nucleus; PF, parafascicular thalamic nucleus; Po, posterior thalamic nucleus; PV, trigeminal principal nucleus; spV, spinal trigeminal tract; SpVc, caudal subnucleus of the spinal trigeminal nucleus; VPM, posteromedial ventral thalamic nucleus; VPMpc, parvicellular part of the posteromedial ventral thalamic nucleus.

Fig. 4 – Composite map of 9 centers of WGA-HRP injection (A) and photomicrograph of a coronal section showing the injection site (Case 12) (B). Numbering of injection centers indicates case number. White, hatched, and gray circles indicate injection centers for the thermal, tactile, and taste regions. The coronal levels from the bregma are shown for each schematic drawing. Scale in B, 1 mm. For abbreviations, see Fig. 3.

Fig. 5 – Distributions of retrograde labels in the dorsal portions of the contralateral PV
and its surroundings after tracer injections into the thalamic regions responding to thermal (A, Case 12), tactile (B, Case 15), and taste (C, Case 18) stimulation of the tongue. Single dots indicate two retrograde labeled cells in (A) and (C), and 10 labeled cells in (B). The coronal levels from the bregma are shown for each schematic drawing in B. For abbreviations, see Fig. 2.

Fig. 6 – Retrograde labels in the dorsal portion of the contralateral PV (-9.8 mm from the bregma) (A) and the superficial portion of the contralateral SpVc at the obex level (B) resulting from WGA-HRP injection into the thermal responsive site in the thalamus shown in Fig. 4B (Case 12). Scale, 0.2 mm in A, and 0.05 mm in B. mcp, middle cerebellar peduncle; PV, trigeminal principal nucleus; spV, spinal trigeminal tract; SpVc, caudal subnucleus of the spinal trigeminal nucleus.

Fig. 7 – Distribution patterns of neural activities responding to thermal stimulation of the tongue in the right SpVc (A and B), and two examples of thermal responses (C). Recording sites of thermal responses (open circles) and sites with no thermal responses (dots) from the four rats were superimposed on the dorsal surface of the caudal medulla (A). The horizontal axis shows distance from the midline. The rostrocaudal reference level is the obex. Distributions of recording sites in one rat which received tracer injection are shown in (B). Two star marks with arrows “a” and “b” indicate injection sites of tracer. Thermal responses recorded with a tracer-containing electrode immediately before injection are shown in “a” and “b” in (C). “v” in (B) indicates a large blood vessel on the dorsal surface of the caudal medulla. W and C indicate application of warm (40°C) and cold (20°C) water.
Fig. 8 – Tracer injection site in the SpVc (A), and anterograde labels in the dorsal region of the PV (Ba), and in the dorsomedial portion of the VPM and dorsolateral region of the VPMpc (C). The injection site corresponds to “a” in Fig. 7B. Nissl-stained section next to the section shown in Ba is shown in Bb. Arrows in Ba and Bb show corresponding blood vessels. Scale, 0.5 mm in A and 0.2 mm in B and C. cc, central canal; Cu, cuneate nucleus; Gr, gracile nucleus; spV, spinal trigeminal tract; SpVc, caudal subnucleus of the spinal trigeminal nucleus. For other abbreviations, see Figs. 2 and 3.